Vitamin Supplementation Reduces Blood Homocysteine Levels

A Controlled Trial in Patients With Venous Thrombosis and Healthy Volunteers

Martin den Heijer, Ingeborg A Brouwer, Gerard M J Bos, Henk J Blom, Nathalie M J van der Put, Anja P Spaans, Frits R Rosendaal, Chris M G Thomas, Hans L Haak, Pierre W Wijermans, Wim B J Gerrits

Abstract—Hyperhomocysteinemia is a risk factor for atherosclerosis and thrombosis and is inversely related to plasma folate and vitamin B12 levels We assessed the effects of vitamin supplementation on plasma homocysteine levels in 89 patients with a history of recurrent venous thrombosis and 227 healthy volunteers Patients and hyperhomocysteinemic (homocysteine level >16 μmol/L) volunteers were randomized to placebo or high-dose multivitamin supplements containing 5 mg folic acid, 0 4 mg hydroxycobalamin, and 50 mg pyridoxine A subgroup of volunteers without hyperhomocysteinemia was also randomized into three additional regimens of 5 mg folic acid, 0 5 mg folic acid, or 0 4 mg hydroxycobalamin Before and after the intervention period, blood samples were taken for measurements of homocysteine, folate, cobalamin, and pyridoxal-5′-phosphate levels Supplementation with high-dose multivitamin preparations normalized plasma homocysteine levels (≤16 μmol/L) in 26 of 30 individuals compared with 7 of 30 in the placebo group Also in normohomocysteinemic subjects, multivitamin supplementation strongly reduced homocysteine levels (median reduction, 30%, range, −22% to 55%) In this subgroup the effect of folic acid alone was similar to that of multivitamin median reduction, 26%, range, −2% to 52% for 5 mg folic acid and 25%, range, −54% to 40% for 0 5 mg folic acid Cobalamin supplementation had only a slight effect on homocysteine lowering (median reduction, 10%, range, −21% to 41%) Our study shows that combined vitamin supplementation reduces homocysteine levels effectively in patients with venous thrombosis and in healthy volunteers, either with or without hyperhomocysteinemia Even supplementation with 0 5 mg of folic acid led to a substantial reduction of blood homocysteine levels ***(Arterioscler Thromb Vasc Biol. 1998;18:356-361.)***

Key Words: homocysteine ■ vitamin supplementation ■ venous thrombosis ■ folate ■ MTHFR

Subjects with hyperhomocysteinemia have a twofold to threefold increase in risk of developing cardiovascular disease or venous thrombosis [1-5] Reduction of plasma homocysteine levels by vitamins may therefore be of major clinical importance Several studies have investigated the homocysteine-lowering properties of pyridoxine (vitamin B6), hydroxycobalamin (vitamin B12), or folic acid alone or in combination [6-11] However, some of these studies were not placebo controlled, and therefore, they cannot distinguish the extent to which the observed effects were due to regression to the mean Other studies were restricted to hyperhomocysteinemic subjects, healthy volunteers, or certain subgroups (eg, elderly people, men, women, or patients with cardiovascular disease or renal failure)

The aim of our study was to estimate and compare the homocysteine-lowering effect of vitamin supplementation in patients with hyperhomocysteinemia-related disease and in healthy volunteers with or without elevated homocysteine levels Therefore, we studied the effects of an 8-week daily combined administration of 5 mg folic acid 0 4 mg hydroxycobalamin, and 50 mg pyridoxine versus placebo on blood homocysteine levels in patients with a history of recurrent venous thrombosis and healthy volunteers We also compared this high-dose multivitamin regimen with single-vitamin regimens of folate or hydroxycobalamin to assess which vitamin at which dose was the most effective in lowering homocysteine levels For reasons of sample size, we restricted this "drug- and dose-finding study" to volunteers with normohomocysteinemia Finally, we analyzed the influence of initial homocysteine and vitamin concentrations and of the 677C→T mutation in the methylenetetrahydrofolate reductase (MTHFR) gene on the homocysteine-lowering effect of multivitamin supplementation

Received August 5 1997, revision accepted October 29 1997

From the Departments of Hematology (M den H I A B H L H P W W W B J G) and Clinical Chemistry (A P S) Leyenburg Hospital The Hague the Department of Hematology Daniel den Hoed Clinic Rotterdam (G M J B) the Department of Pediatrics Laboratory of Pediatrics and Neurology (H J B , N M J van der P) and the Department of Obstetrics and Gynecology Laboratory of Endocrinology and Reproduction (C M G T) University Hospital Nijmegen Nijmegen and Departments of Clinical Epidemiology and Hematology (F R R) University Hospital Leiden Leiden the Netherlands

Correspondence to Martin den Heijer MD PhD Department of General Internal Medicine University Hospital Nijmegen PO Box 9101 6500 HB Nijmegen the Netherlands

E mail m denheijer@aig azn nl

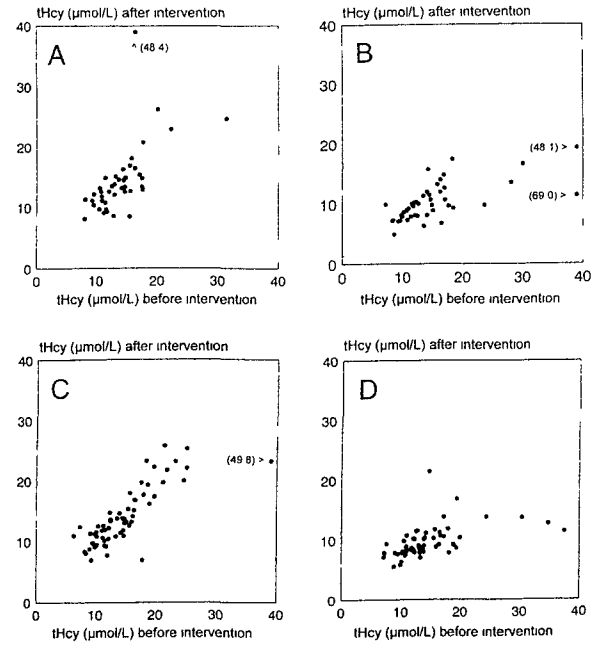
Methods

The study group was recruited from subjects who had participated in a previous case-control study [4] In the current study, 92 of the 185 patients with recurrent venous thrombosis and 230 of the 500 volunteers from the general population agreed to participate in an 8-week daily vitamin supplementation trial Six participants (three from each group) withdrew during the study All participants were asked not to take ("self-prescribed") vitamin supplements for at least 2 months prior to the start of the current study

Both patients and hyperhomocysteinemic healthy volunteers were randomized to either a placebo or a high-dose multivitamin schedule Each multivitamin tablet contained 5 mg folic acid, 0 4 mg hydroxycobalamin, and 50 mg pyridoxine (Randomization of the volunteers had been stratified by homocysteine level in a previous study[4] [cutoff point, 16 μmol/L]) Volunteers with previous homocysteine levels ≤16 μmol/L were randomized to placebo, multivitamin, or single-vitamin regimens (5 mg folic acid, 0 5 mg folic acid, or 0 4 mg hydroxycobalamin) These three additional subregimens were restricted to normocysteinemic volunteer group because the other subgroups were too small to allow randomization into more than two schedules Randomization was performed by using the last digit of each patient's number So all hyperhomocysteinemic subjects and normocysteinemic patients with recurrent venous thrombosis with an odd or even number were assigned to the multivitamin or placebo group, respectively In the normohomocysteinemic healthy volunteers, subjects with a last digit of 0 or 1 were assigned to placebo, 2 and 3, to multivitamins, 4 and 5, to 5 mg folic acid,, 6 and 7, to 0 5 mg folic acid, and 8 and 9, to 0 4 mg vitamin B12 All subjects were asked to take 1 tablet per day for 56 days The trial was kept double-blind Before and after the supplementation period, blood was collected after an overnight fast for homocysteine, folate, cobalamin, and pyridoxal-5′-phosphate measurements

For homocysteine and vitamin measurements, blood samples were taken from the antecubital vein and collected into EDTA-containing tubes Whole blood was stored at −70°C for pyridoxal-5′-phosphate determination For the other determinations, EDTA-treated samples were immediately placed on ice and centrifuged within half an hour at 2000*g* for 10 minutes The plasma was separated and stored at −20°C The EDTA-treated samples for folate and cobalamin measurements were stored at −70°C and analyzed within 2 months Folate and cobalamin concentrations were measured with a Dualcount SPNB (solid phase no boil) radioassay kit (Diagnostic Products Corp) Determination of pyridoxal-5′-phosphate was performed by high-performance liquid chromatography techniques according to Schrijver et al[12] with some modifications [13] Total homocysteine concentrations were measured according to the method described by Fiskerstrand et al[14] with some modifications [15] Mutation analysis was carried out by means of polymerase-chain reaction and restriction enzyme digestion as described elsewhere [16]

In the analysis we first looked at normalization rates of homocysteine levels after multivitamin or placebo supplementation Therefore, we calculated the fraction of hyperhomocysteinemic subjects (homocysteine >16 μmol/L in the present study) who became normohomocysteinemic (homocysteine ≤16 μmol/L) after the supplementation period The cutoff point was a rounded value based on the 80th percentile in our previous study [4] Second, we calculated the percent homocysteine reduction for each subject and compared the median reduction in the different vitamin supplementation groups with respect to the corresponding placebo group To compare the homocysteine-lowering effects in patients and volunteers, we later stratified the patients into a hyperhomocysteinemic and a normohomocysteinemic group according to their homocysteine levels as determined in our previous study (cutoff point, 16 μmol/L) Finally, we studied determinants of the homocysteine-lowering effect of multivitamin supplementation by calculating the median reduction in men and women, in subjects under or above 53 years of age (median age of healthy volunteers), and for several strata (tertiles) of initial homocysteine, folate, cobalamin, and pyridoxal-5′-phosphate concentrations, as well as for the three different MTHFR-genotypes (677C→T) To evaluate the difference in median reduction, we used the Mann-Whitney *U* test for unpaired cases (SPSS software) All participants gave their informed, written consent, and the study protocol was approved by the medical ethics committee of Leyenburg Hospital



Total plasma homocysteine (tHcy) levels before (*x* axis) and after (*y* axis) 8 weeks of daily multivitamin or placebo supplementation in patients with a history of recurrent venous thrombosis (A, placebo group, B, multivitamin group) and in healthy volunteers (C, placebo group, D, multivitamin group) The multivitamin tablets contained 5 mg folic acid, 0.4 mg hydroxycobalamin, and 50 mg pyridoxine

Results

The median age of the patient group was 62 years (range, 31 to 89) and of the volunteers, 53 years (range, 23 to 82) The median homocysteine concentration of the patient group was 13 6 μmol/L (range, 7 2 to 69) and of the volunteer group 12 8 μmol/L (range, 4 7 to 49 8)

The Figure shows the homocysteine concentrations before and after intervention for the placebo and high-dose multivitamin groups of both patients and volunteers In the multivitamin group, 11 of 14 hyperhomocysteinemic patients with thrombosis had a normalized value after intervention (cutoff point, 16 μmol/L) compared with only 4 out 10 in the placebo group A very similar observation was made in the healthy volunteers

In the multivitamin group, 15 of 16 hyperhomocysteinemic subjects had a normalized value (cutoff point, 16 μmol/L) compared with only 3 of 20 in the placebo group So for all hyperhomocysteinemic individuals together, 26 of 30 subjects had normalized homocysteine levels (<16 μmol/L) after supplementation with multivitamins compared with only 7 of 30 in the placebo group

Table 1 shows that there was no clear diffe ence between patients with recurrent venous thrombosis and healthy volunteers with respect to their homocysteine-lowering response due to multivitamin supplementation In both groups there was also a substantial effect in the normohomocysteinemic subjects

TABLE 1. Homocysteine-Lowering Effect of Multivitamin or Placebo Supplementation in Patients With Recurrent Venous Thrombosis and in Healthy Volunteers

Study Subjects	n	Median tHcy Before Intervention, μmol/L (Range)	Median tHcy After Intervention, μmol/L (Range)	Median Reduction, % (Range)
Patients with recurrent venous thrombosis	89			
Homocysteine >16 μmol/L*	34			
Placebo	15	15 8 (10 6–31 5)	14 8 (12 6–26 3)	−3 (−30–27)
Multivitamin†	19	16 5 (10 7–69 0)	10 7 (6 8–17 5)	36 (4–83)‡
Homocysteine ≤16 μmol/L*	55			
Placebo	28	12 8 (8 1–17 5)	12 2 (8 1–48 4)	−1 (−193–45)
Multivitamin†	27	11 8 (7 2–48 1)	8 6 (4 9–19 3)	20 (−38–60)‡
Healthy volunteers	227			
Homocysteine >16 μmol/L*	50			
Placebo	27	18 0 (9 9–49 8)	17 4 (9 2–25 8)	0 (−27–53)
Multivitamin†	23	16 6 (11 0–37 5)	10 7 (7 0–21 4)	36 (−46–70)‡
Homocysteine ≤16 μmol/L*	177			
Placebo	36	11 5 (6 4–17 8)	11 4 (7 0–21 4)	3 (−72–61)
Multivitamin†	34	11 8 (7 1–19 3)	8 5 (5 5–11 5)	30 (−22–55)‡
Single-vitamin regimen	107			

tHcy indicates total plasma homocysteine The homocysteine lowering effect is expressed as the median percent reduction in homocysteine concentration after intervention

*Stratified on homocysteine levels in the previous study

†Containing 5 mg folic acid 0 4 mg hydroxycobalamin and 50 mg pyridoxine

‡$P<$ 001 compared with the corresponding placebo group

In Table 2 we compared the homocysteine-lowering effect of several single-vitamin regimens in volunteers who were normohomocysteinemic in a previous study From these data we may conclude that the effect of 5 mg folic acid, even a low dose of 0 5 mg, is nearly as effective as the multivitamin regimen In contrast, vitamin B12 only slightly decreased the homocysteine concentration

In Table 3 we analyzed the effects of age and sex with respect to the homocysteine-lowering effect of multivitamin supplementation For reasons of homogeneity, we restricted this analysis to volunteers who had been randomized into either the placebo or the multivitamin group (n=120) We found a similar homocysteine-lowering effect in men and women and in subjects under and above 53 years of age

In Table 4 we stratified the homocysteine-lowering effect on tertiles of initial homocysteine, folate, cobalamin, and pyridoxal-5′-phosphate levels We found a stronger homocysteine-lowering effect in subjects with high initial homocysteine levels However, even in subjects with an initial homocysteine level of <11 8 μmol/L, we still found a median reduction of 21% (range, −22 to 41%) An inverse effect was seen with respect to initial vitamin concentrations The homocysteine-lowering effect was strongest in subjects with low folate, cobalamin, or pyridoxal-5′-phosphate concentrations

Six of the 92 patients with recurrent venous thrombosis were homozygous for the 677C→T mutation versus 22 of the 230 control subjects (odds ratio, 0 7, 95% confidence interval, 0 3 to 1 7) In Table 4 we also show that the homocysteine-lowering effect of multivitamin supplementation was not

TABLE 2. Homocysteine-Lowering Effect of Several Vitamin Regimens or Placebo in Normohomocysteinemic Healthy Volunteers

Regimens	n	Median tHcy Before Intervention, μmol/L (Range)	Median tHcy After Intervention, μmol/L (Range)	Median Reduction, % (Range)
Placebo	36	11 5 (6 4–17 8)	11 4 (7 0–18 0)	3 (−72–61)
Multivitamin*	34	11 8 (7 1–19 3)	8 5 (5 5–11 5)	30 (−22–55)†
Folic acid 5 mg	35	11 8 (7 0–22 1)	8 7 (5 9–13 8)	26 (−2–52)†
Folic acid 0 5 mg	36	12 2 (4 7–22 3)	10 0 (2 8–13 8)	25 (−54–40)†
Hydroxycobalamin 0 4 mg	36	12 6 (6 4–18 4)	11 0 (6 7–15 9)	10 (−21–41)‡

tHcy indicates total plasma homocysteine The homocysteine-lowering effect is expressed as the median percent reduction in homocysteine concentration after intervention

*Containing 5 mg folic acid 0 4 mg hydroxycobalamin and 50 mg pyridoxine

†$P<$ 001 compared with the placebo group

‡$P=$ 14

TABLE 3. Homocysteine-Lowering Effect of Multivitamin or Placebo Supplementation in Men and Women and in Subjects <53 or ≥53 Years Old

Study Subjects	n	Median tHcy Before Intervention, μmol/L (Range)	Median tHcy After Intervention, μmol/L (Range)	Median Reduction, % (Range)
Men				
Placebo	24	14 8 (7 4–49 8)	13 2 (7 0–25 3)	12 (−69–61)
Multivitamin*	26	14 0 (7 2–37 5)	10 1 (7 6–21 4)	33 (−46–70)
Women				
Placebo	39	12 4 (6 4–21 8)	12 7 (7 0–25 8)	−1 (−72–25)
Multivitamin*	31	12 1 (7 1–34 8)	8 7 (5 5–16 8)	31 (−22–64)
Volunteers <53 years				
Placebo	27	12 0 (6 4–49 8)	10 9 (7 0–25 8)	5 (−72–61)
Multivitamin*	23	13 1 (7 1–34 8)	8 3 (5 5–21 4)	31 (−46–64)
Volunteers ≥53 years				
Placebo	36	14 7 (7 4–25 1)	13 9 (9 5–25 3)	−1 (−69–19)
Multivitamin*	34	13 8 (9 5–37 5)	9 5 (6 3–16 8)	32 (3–70)

tHcy indicates total plasma homocysteine The homocysteine lowering effect is expressed as the median percent reduction in homocysteine concentration after intervention

*Containing 5 mg folic acid 0 4 mg hydroxycobalamin and 50 mg pyridoxine

impaired in subjects homozygous for the 677C→T mutation and in fact, might even be stronger

Discussion

We investigated the effect of multivitamin supplementation on homocysteine levels in patients with recurrent venous thrombosis and healthy volunteers from the general population We found that combined supplementation with folic acid, hydroxycobalamin, and pyridoxine effectively reduced and normalized homocysteine levels in patients with recurrent venous thrombosis as well as in healthy volunteers For a subgroup of normohomocysteinemic volunteers, folic acid at a dose of 5 mg or even 0 5 mg seemed to be almost as effective as the high-dose multivitamin supplementation Oral cobalamin supplementation had only a moderate effect on homocysteine levels

In 1985 Brattstrom et al[6] reported a substantial homocysteine reduction in 15 volunteers who received 5 mg folic acid per day for 4 weeks Wilcken et al[7] reported a homocysteine-lowering effect of folic acid supplementation in patients with chronic renal insufficiency Franken et al[8] and van den Berg et al[9] reported significant reductions in postmethionine-loading homocysteine concentrations with vitamin B6 folic acid, or a combination of both in patients with vascular disease Although these studies were performed in large groups of patients, they were not placebo controlled and were restricted to hyperhomocysteinemic subjects, which characteristics make them rather sensitive to regression to the mean Ubbink et al[10] studied the effects of 1 mg folic acid, 0 4 mg cyanocobalamin, and 12 2 mg pyridoxal HCl alone or in combination in a placebo-controlled study in subjects with hyperhomocysteinemia High-dose multivitamin administration resulted in a 49 8% reduction of the mean homocysteine level Naurath et al[11] studied the effect of intramuscular vitamin supplementation with folate, vitamin B6, and vitamin B12 in elderly subjects with blood vitamin concentrations in the normal range

In our study we were able to compare the effects in patients with homocysteine-related disease (venous thrombosis) with those in healthy volunteers These effects were quite similar We also found about the same effects in men compared with women and in subjects under and above 53 years of age The strongest homocysteine-lowering effect of vitamin supplementation was seen in subjects with high initial homocysteine and/or low initial vitamin concentrations However, we also observed a moderate reduction in homocysteine levels in subjects with homocysteine and vitamin levels within the normal range This observation raises the question of "normal" homocysteine and vitamin levels Our definition of hyperhomocystinemia at the 80th percentile of the distribution in the general population is arbitrary Other suggested definitions are based on mean concentrations in populations without cardiovascular disease [17] at the flat plateau of the homocysteine-folate plot [18] We think that the best definition should be based on clinical intervention studies the lowest concentration at which vitamin supplementation reduces the risk of vascular disease However, data on clinical studies are not yet available

In a subgroup of normohomocysteinemic volunteers, we found approximately the same homocysteine-lowering effect of folic acid at a dose of 0 5 mg as with a dose of 5 mg This means that considerably low doses of folic acid supplementation are effective in lowering homocysteine levels Further studies are needed to see whether doses lower than 0 5 mg may also be effective

Although folic acid supplementation seems to be the cornerstone in the treatment of hyperhomocysteinemia, there are some reasons for adding cobalamin and pyridoxine First, this combination may have a stronger effect in subjects with low cobalamin or pyridoxine levels Second, folate administration alone might mask vitamin B12 deficiency Addition of cobalamin in sufficient dose prevents the complications of vitamin B12 deficiency, such as subacute combined degeneration of the spinal cord, even in the case of pernicious anemia [19] We did

TABLE 4. Homocysteine-Lowering Effect of Multivitamin or Placebo Supplementation Stratified Over Tertiles of Homocysteine, Folate, Cobalamin, and Pyridoxal-5′-Phosphate Levels in 120 Healthy Volunteers

	n	Median tHcy Before Intervention, μmol/L (Range)	Median tHcy After Intervention, μmol/L (Range)	Median Reduction, % (Range)
Placebo				
Homocysteine				
<11 8 μmol/L	21	9 9 (6 4–11 6)	10 7 (7 0–12 7)	−3 (−72–25)
11 8–15 7 μmol/L	20	14 1 (11 8–15 6)	13 2 (7 8–15 4)	9 (−19–35)
≥15 7 μmol/L	22	18 9 (15 7–49 8)	19 5 (7 0–25 8)	−1 (−27–61)
Folate				
<10 4 nmol/L	19	16 6 (9 3–49 8)	16 2 (7 0–25 8)	0 (−21–61)
10 4–14 3 nmol/L	23	12 5 (6 4–25 1)	12 7 (8 8–25 3)	−1 (−72–21)
≥14 3 nmol/L	21	12 0 (7 4–25 1)	12 5 (7 8–22 1)	5 (−69–35)
Vitamin B12				
<207 pmol/L	20	14 0 (9 1–49 8)	12 9 (9 2–25 3)	−6 (−25–53)
207–296 pmol/L	23	13 6 (7 4–21 8)	13 8 (7 8–25 8)	−2 (−69–35)
≥296 pmol/L	20	14 4 (6 4–25 1)	12 5 (7 0–23 2)	9 (−72–61)
Vitamin B6				
<37 nmol/L	18	11 9 (6 4–21 8)	11 5 (8 4–23 3)	−2 (−72–18)
37–47 nmol/L	21	14 6 (8 4–24 6)	12 7 (7 0–25 8)	0 (−21–25)
>47 nmol/L	24	15 0 (7 4–49 8)	13 6 (7 0–25 3)	9 (−69–61)
MTHFR 677 C→T				
+/+	5	16 4 (9 0–49 8)	13 3 (7 0–23 2)	12 (2–61)
+/−	25	14 6 (9 3–25 1)	13 7 (7 0–25 8)	0 (−27–25)
−/−	33	12 4 (6 4–25 1)	12 6 (7 8–22 1)	−1 (−72–35)
Multivitamin*				
Homocysteine				
<11 8 μmol/L	18	10 3 (7 1–11 7)	7 8 (5 5–10 7)	21 (−22–41)
11 8–15 7 μmol/L	21	13 2 (11 8–15 5)	9 0 (7 0–21 4)	31 (−46–47)
≥15 7 μmol/L	18	18 5 (15 7–37 5)	11 0 (7 0–25 8)	44 (13–70)
Folate				
<10 4 nmol/L	19	15 7 (9 9–37 5)	10 2 (5 8–21 4)	39 (−46–70)
1 04–14 3 nmol/L	18	12 2 (7 2–18 8)	8 7 (6 3–11 8)	27 (−8–51)
≥14 3 nmol/L	20	12 4 (7 1–24 4)	8 9 (5 5–13 8)	27 (−22–44)
Vitamin B12				
<207 pmol/L	19	14 7 (9 9–37 5)	10 3 (5 8–21 4)	34 (−46–70)
207–296 pmol/L	18	12 7 (7 6–24 4)	8 6 (6 3–16 8)	31 (−22–55)
≥296 pmol/L	20	12 0 (7 1–18 9)	8 6 (5 5–13 8)	26 (−8–57)
Vitamin B6				
<37 nmol/L	14	14 7 (7 1–34 8)	8 9 (6 3–13 7)	40 (0–74)
37–47 nmol/L	24	13 3 (8 8–37 5)	8 5 (5 5–16 8)	34 (7–70)
>47 nmol/L	19	12 3 (7 2–24 4)	9 3 (7 8–21 4)	20 (−46–51)
MTHFR 677C→T				
+/+	8	16 4 (10 9–37 5)	9 7 (7 0–13 7)	49 (3–70)
+/−	21	12 6 (7 1–34 8)	8 6 (6 3–21 4)	2[illegible] (−46–64)
−/−	28	13 1 (8 8–24 4)	8 8 (5 5–16 8)	31 (7–57)

tHcy indicates tal plasma homocysteine, MTHFR, methylenetetrahydrofolate reductase The homocysteine-lowering effect is expressed as the percent reduction in homocysteine concentration after intervention

*Containing 5 mg folic acid, 0 4 mg hydroxycobalamin, and 50 mg pyridoxine

not study doses higher than 50 mg pyridoxine because higher doses may cause sensory neuropathy [20,21]

Recently, we found the 677C→T mutation in the MTHFR gene [16,22,23] This mutation is associated with elevated homocysteine levels, but it is unclear whether this mutation is also associated with arterial vascular disease [24] In this study the prevalence of the 677C→T mutation in the recurrent venous thrombosis group did not really differ from that in the control group This finding is in accordance with the results in a study of first-time venous thrombosis [25] We found that the homocysteine-lowering effect in subjects with this mutation en might be even stronger than in those without this mutation This finding is in accordance with the study of Malinow et al,[26] These results suggest that the effect of a mutated MTHFR might be "compensated" by a higher folate intake

In conclusion, our study shows that combined supplementation with folic acid, cobalamin, and pyridoxine reduces homocysteine levels by ≈30% compared with placebo within 8 weeks in patients with recurrent venous thrombosis as well as in healthy volunteers. Whether the reduction in homocysteine levels by vitamin supplementation will lead prevention of arterial vascular disease and venous thrombosis is a major task for further clinical research.[27,28]

Acknowledgments

This work was supported by grants from the Prevention Fund (to G.M.J.B.) (28–2263-1). We thank G E Th Ferguson, pharmacist, for preparing the vitamin and placebo tablets and C Postma, Y Lenstra, M.T W B te Poele-Pothoff, A de Graaf-Hess, D van Oppenraaij-Emmerseel, M.F.G. Segers, L M.F Geelen, and J Beunk for their excellent laboratory assistance We wish thank R Clarke, MD, MRCP, for his valuable comments of a previous version of the manuscript Finally, we thank all participants for their kind contribution the study

References

1 Ueland PM, Refsum H, Brattstrom L Plasma homocysteine and cardiovascular disease In Francis RB, Jr, ed *Atherosclerotic Cardiovascular Disease, Hemostasis, and Endothelial Function* New York/Basel/Hong Kong Marcel Dekker Inc, 1993 183–236

2 Rees MM, Rodgers GM Homocysteinemia association of a metabolic disorder with vascular disease and thrombosis *Thromb Res* 1993,71 337–359

3 Boushey CJ, Beresford SAA, Omenn GS, Motulsky AG A quantitative assessment of plasma homocysteine as a risk factor for vascular disease probable benefits of increasing folic acid intakes *JAMA* 1995,274 1049–1057

4 den Heijer M, Blom HJ, Gerrits WBJ, Rosendaal FR, Haak HL, Wijermans PW, Bos GMJ Is hyperhomocysteinaemia a risk factor for recurrent venous thrombosis? *Lancet* 1995,345 882–885

5 den Heijer M, Koster T, Blom HJ, Bos GMJ, Briet E, Reitsma PH, Vandenbroucke JP, Rosendaal FR Hyperhomocysteinemia as a risk factor for deep-vein thrombosis *N Engl J Med* 1996,334 759–762

6 Brattstrom LE, Hultberg BL, Hardebo JE Folic acid responsive postmenopausal homocysteinemia *Metabolism* 1985,34 1073–1077

7 Wilcken DEL, Dudman NPB, Tyrrell PA, Robertson MR Folic acid lowers elevated plasma homocysteine in chronic renal insufficiency possible implications for prevention of vascular disease *Metabolism* 1988, 37 697–701

8 Franken DG, Boers GHJ, Blom HJ, Trijbels FJM, Kloppenborg PWC Treatment of mild hyperhomocysteinemia in vascular disease patients *Arterioscler Thromb* 1994,14 465–470

9 van den Berg M, Franken DG Boers GHJ Blom HJ, Jakobs C Stehouwer CDA, Rauwerda JA Combined vitamin B6 plus folic acid therapy in young patients with arteriosclerosis and hyperhomocysteinemia *J Vasc Surg* 1994,20 933–940

10 Ubbink JB, Vermaak WJH, van der Merwe A, Becker PJ Delport R, Potgieter HC Vitamin requirements for the treatment of hyperhomocysteinemia in humans *J Nutr* 1994,124 1927–1933

11 Naurath HJ, Joosten E, Riezler R, Stabler SP, Allen RH, Lindenbaum J Effects of vitamin B12, folate, and vitamin B6 supplements in elderly people with normal serum vitamin concentrations *Lancet* 1995,346 85–89

12 Schrijver J, Speek AJ, Schreurs WHP Semi-automated fluorometric determination of pyridoxal-5′-phosphate (vitamin B6) in whole blood by high-performance liquid chromatography (HPLC) *Int J Vitam Nutr Res* 1981, 51 216–222

13 Steegers-Theunissen RPM, Boers GHJ, Steegers EAP, Trijbels FJM, Thomas CMG, Eskes TKAB Effects of sub-50 oral contraceptives on homocysteine metabolism a preliminary study *Contraception* 1992,45 129–139

14 Fiskerstrand T, Refsum H, Kvalheim G, Ueland PM Homocysteine and other thiols in plasma and urine automated determination and sample stability *Clin Chem* 1993,39 263–271

15 te Poele-Pothoff MTWB, van der Berg M, Franken DG, Boers GHJ, Jacobs C, de Kroon IFI, Eskes TAKB, Trijbels JMF, Blom HJ Three different methods for the determination of tal homocysteine in plasma *Ann Clin Biochem* 1995,32 218–220

16 Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers, GHJ, den Heijer M, Kluytmans LAJ, van den Heuvel LP, Rozen R A candidate genetic risk factor for vascular disease a common mutation in methylene-tetrahydrofolate reductase *Nat Genet* 1995,10 111–113

17 Ubbink JB, Vermaak WJH, Bennett JM, Becker PJ, van Staden DA, Bissbort S The prevalence of homocysteinemia and hypercholesterolemias in angiographically defined coronary heart disease *Klin Wochenschr* 1991, 69 527–534

18 Selhub J, Jaques PF, Wilson PWF, Rush D, Rosenberg IH Vitamin status and intake as primary determinants of homocysteinemia in an elderly population *JAMA* 1993,270 2693–2698

19 Lederle FA Oral cobalamin for pernicious anemia medicine's best kept secret? *JAMA* 1991,265 94–95

20 Parry GJ, Bredensen DE Sensory neuropathy with low dose pyridoxine *Neurology* 1985,35 1466–1468

21 Schaumberg H, Kaplan J, Windebanke A, Vick N, Rasmus S, Pleasure D, Brown MJ Sensory neuropathy from pyridoxine abuse *N Engl J Med* 1983,309 445–448

22 Engbertsen AMT, Franken DG, Boers GHJ, Stevens EMB, Trijbels FJM, Blom HJ Thermolabile 5,10-methylenetetrahydrofolate reductase as a cause of mild hyperhomocysteinemia *Am J Hum Genet* 1995,56 142–150

23 Kluytmans LAJ, van den Heuvel LPWJ, Boers GHJ, Frosst P, Stevens EMB, van Oost BA, den Heijer M, Trijbels FJM, Rozen R, Blom HJ Molecular genetic analysis in mild hyperhomocysteinemia a common mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease *Am J Hum Genet* 1996,58 35–41

24 Kluytmans LAJ, Kastelein JJP, Lindemans J, Boers GHJ, Heil SG, Bruschke AVG, Jukema JW, van den Heuvel LPWJ, Trijbels JMF, Boerma GJM, Verheugt FWA, Willems F, Blom HJ Thermolabile methylenetetrahydrofolate reductase in coronary artery disease *Circulation* 1997,96 2573–2577

25 Kluytmans LAJ, den Heijer M, Reitsma PH, Heil SH, Blom HJ, Rosendaal FR Thermolabile methylenetetrahydrofolate reductase and factor V Leiden in the risk of deep-vein thrombosis *Thromb Haemost* In press

26 Malinow MR, Nieto FJ, Kruger WD, Duell PB, Hess DL, Gluckman RA, Block PC, Holzgang, Anderson PH, Seltzer D, Upson B, Lin QR The effects of folic acid supplementation on plasma tal homocysteine are modulated by multivitamin use and methylenetetrahydrofolate reductase genotypes *Arterioscler Thromb Vasc Biol* 1997,17 1157–1162

27 Stampfer MJ, Rimm EB Folate and cardiovascular disease why we need a trial now *JAMA* 1996,275 1929–1930

28 den Heijer M, Bos GMJ, Gerrits WBJ, Blom HJ Will a decrease of blood homocysteine by vitamin supplementation reduce the risk for vascular disease? *Fibrinolysis* 1994,8(suppl 2) 91–92